

15.06.2022

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→ Properties of added cloning vector

- Should possess its own Ori site.
- — " — be presence of unique restriction sites for corresponding restriction endonucleases.

~~should also possess marker systems~~

~~↓ enhances tract rate~~

→ selective

→ scorable

exogenous DNA with

transformation is uptake

→ Plasmids

Extrachromosomal, double stranded, supercoiled, circular DNA usually found in prokaryotes & eukaryotic yeast.

→ pBR 322 plasmid is

made by ligating 3 naturally occurring plasmids viz ampicillin resistant gene, tetracycline resistant gene & ori

~ 4.4 kb p ~ 4000 bp

→ PUC plasmide is

- possess ampicillin resistant gene, ori site, Lac I gene, promoter, operator, Lac Z' gene, multiple cloning site & terminator
- ~ 2.6 kb p ~ 2600 bp
- Multiple cloning site [MCS] → many unique restriction sites in one particular location on the plasmid
- PUC stands for Plasmid of University of California treated by Joasin Messing and coworkers.
- Mol. size of plasmid is 2686 bp
- It contains Amp<sup>r</sup> as a selective marker
- It shows presence of Lac I gene, promoter sequence, operator site, Lac Z' gene with MCS & terminator sequence
- PUC series plasmids do contain Ori site derived from pMB 1.
- PUC series includes pUC 18, pUC 19, pUC 12 & pUC 13, etc.

→

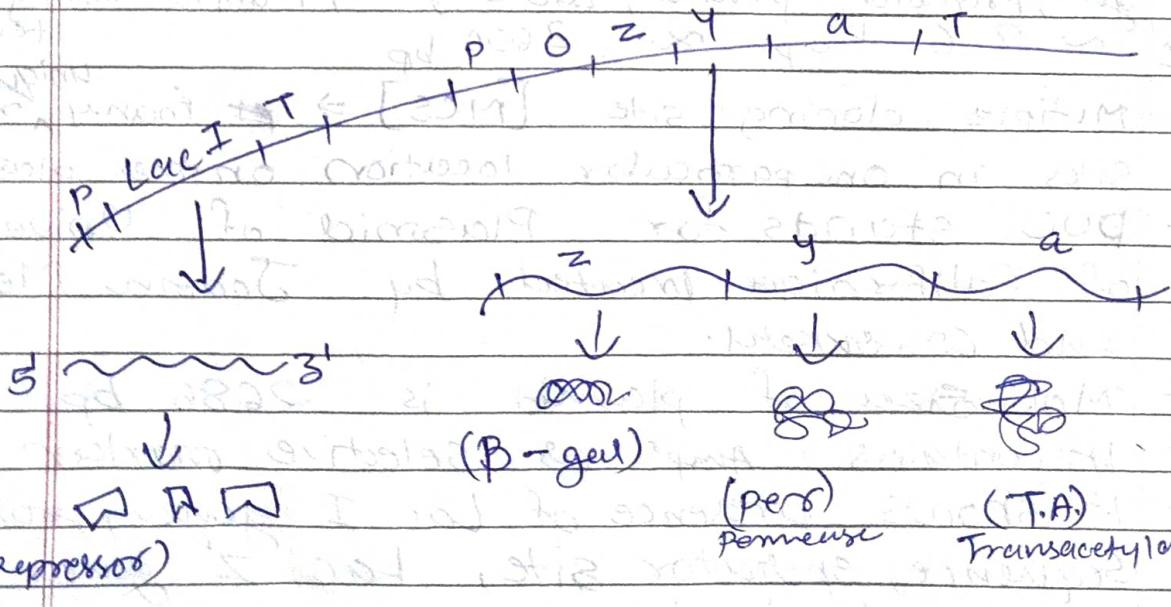
Plasmid DNA

Replisome Episome

- has own origin site for replication which helps it to integrate with host genome & replicate
- has insertion sequence

⇒ Lac Operon

- Defn → operon that has genes whose gene products are involved in lactose metabolism
- Lactose → a carbohydrate compound → galactose & glucose
- bond →  $\beta$ -1,4 glycosidic linkage
- transcribable operon → a cluster of genes that are transcribed together to give a single mRNA.
- Operon → Inducible - expresses in presence of a specific compound amount
- Repressible - represses in presence of a specific compound amount

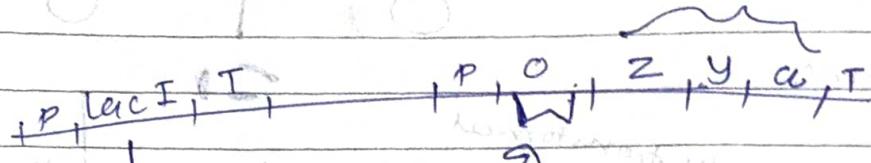


→

Working of Lac operon

→

In absence of Lactose structural genes

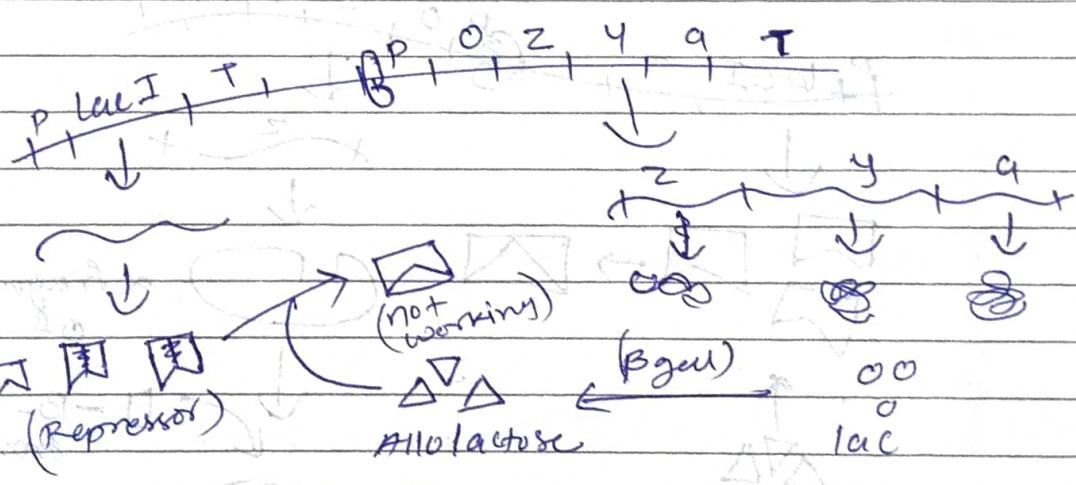


In absence of lactose  
lacI gene gets to express  
itself & produce repressors

that later binds with operon &  
then doesn't allow the structural  
genes (Z, Y & a) to express themself

→

In presence of Lactose:

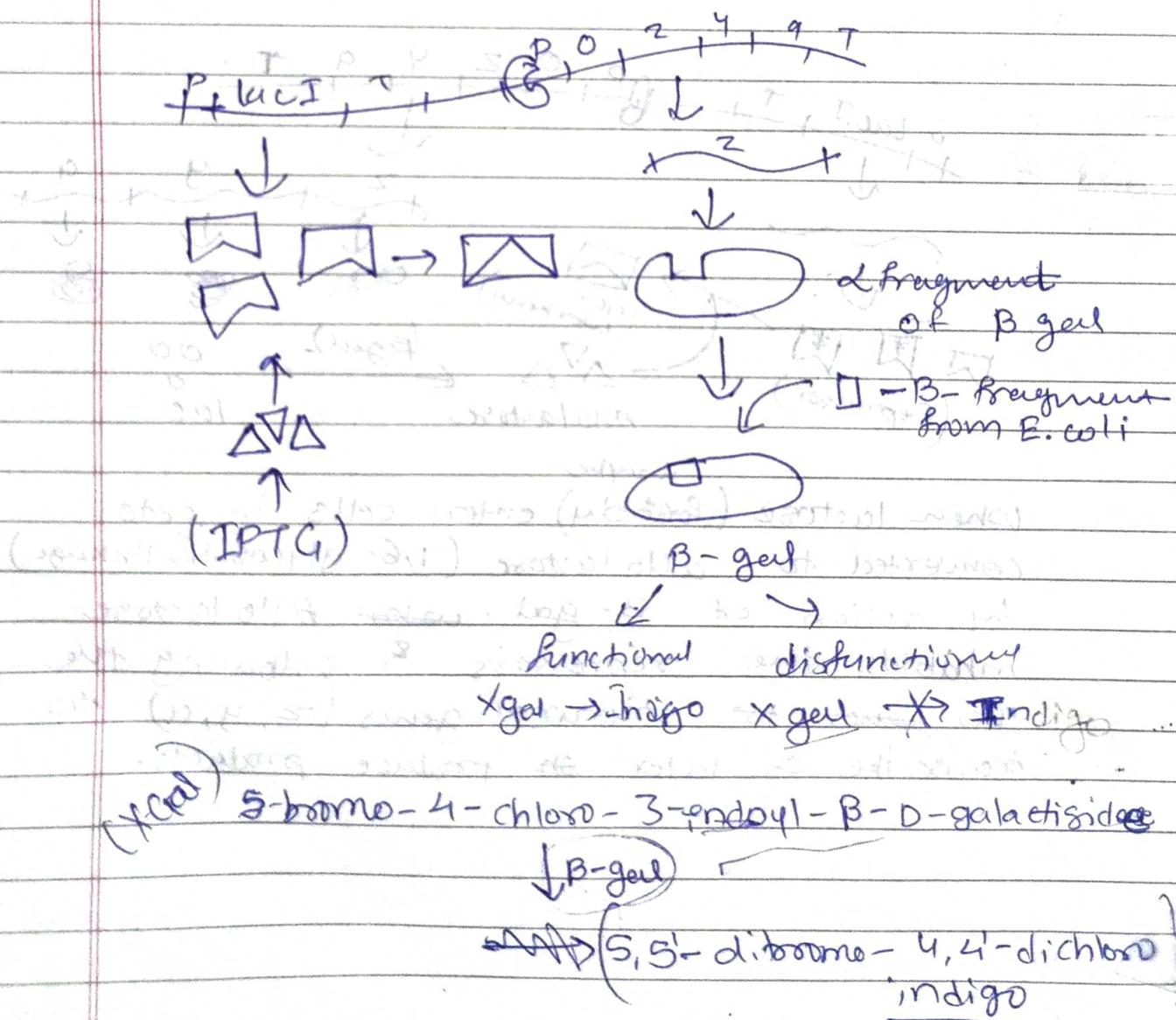
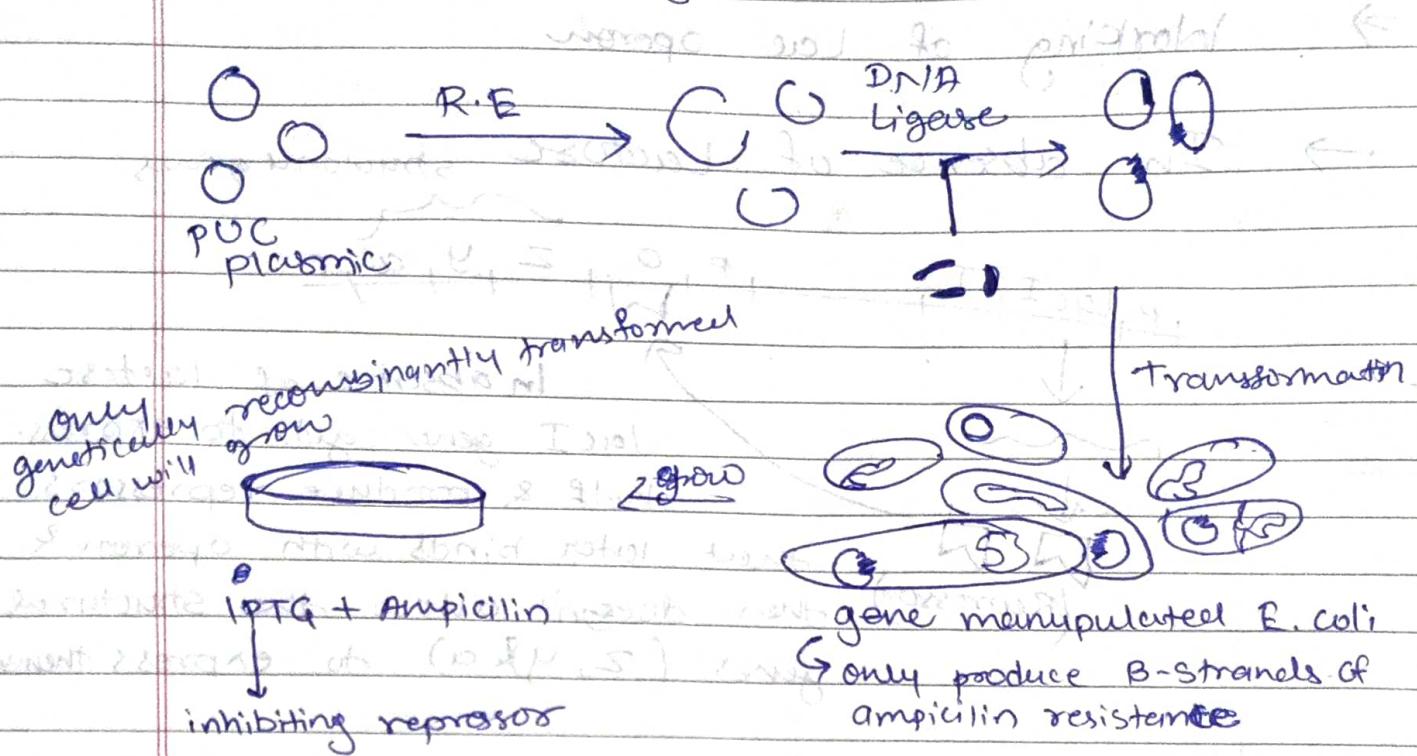


when lactose (lactulose) enters cells it gets converted to allolactose (1,6-glycosidic linkage) by action of  $\beta$ -gal. Allolactose inhibits the repressors & allowing the structural genes (Z, Y, a) to transcribe & later produce products.

$\beta$ -gal

lactose  $\rightarrow$  allolactose  
operator

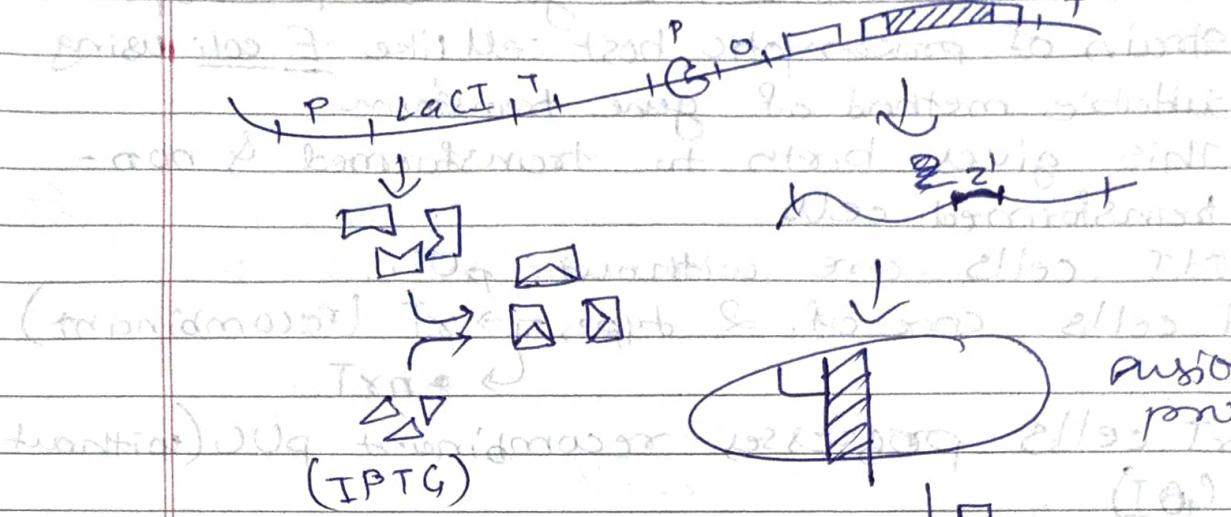
⇒ Blue white screening



⇒ Inside R.T. cell: ~~bioactivity~~ ~~gradual~~ ~~bioactivity~~

bioactivity ~~gradual~~ ~~bioactivity~~ ~~gradual~~ ~~bioactivity~~

bioactivity ~~gradual~~ ~~bioactivity~~ ~~gradual~~ ~~bioactivity~~



→ Advantages  $\rightarrow$  X-gal  $\rightarrow$  Indigo

- Low molecular size

- High copy number

- MCS

- E. coli is easy to work with

⇒ Cloning of foreign DNA in pUC as a plasmid & screening of recombinant

- Consider an intact ds circular pUC series plasmid as a cloning vector

- Treat it with suitable R.E. (restriction enzyme) whose restriction site is present within the MCS

- This gives rise to production of cleaved linear ds pUC

- Ligate it with foreign DNA which has been cleaved with same kind of RE

- In presence of suitable DNA ligase with required co-factor

Ligation give rise to self-ligated pUC, Recombinant pUC with GOI & Recombinant pUC without GOI

- Obtained plasmid DNA are then subjected for transformation into the genetically manipulated strain of prokaryotic host cell like E. coli using suitable method of gene transform.

- This gives birth to transformed & non-transformed cells.

- NT cells are without pUC.

- T. cells are of 2 types  $\rightarrow$   $\alpha$ T (recombinant)  $\downarrow \rightarrow$  nrT

-  $\alpha$ T cells possesses recombinant pUC (without COT).

- nrT cells possesses self ligated pUC.

-  $\alpha$ T & nrT colonies can be differentiated on the same plate of transformation via blue white screening.

- nrT colonies appears blue in colour because such cells of host carry non recombinant pUC where LacZ' gene is intact. On a growth medium supplemented with IPTG, X-gal & ampicillin these cells are genetically manipulated showing deletion mutation within LacZ' gene of Lac operon at chromosomal DNA.

Here IPTG acts as an artificial inducer which stimulates the expression of LacZ' as well as LacZ gene.

Accordingly,  $\alpha$ -fragment complements to the  $\beta$ -fragment to form functional  $\beta$ -gal which convert X-gal to Indigo. Hence, nrT cells appears blue.

However,  $\alpha$ T colonies appear white in colour.

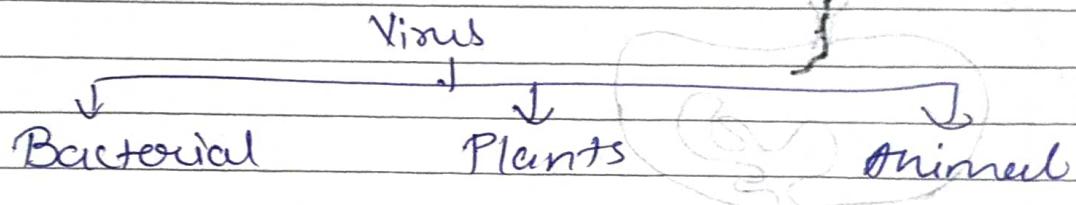
This is due to the fact that, in  $\alpha$ T cells, insertion of foreign DNA into the Xbs.

of LacZ' gene, causes insertionional inactivation of gene. Thus,  $\alpha$ T cells are unable to produce  $\beta$ -fragment of  $\beta$ -gal & eventually to unable to synthesize functional  $\beta$ -gal, unable to convert X-gal to indigo & appears white.

- In presence of ampicillin, NT cells because of lack of pUC an ampicillin resistance never appears.

## ⇒ Viruses:

↳ The ultramicroscopic, disease causing entity that acts as an obligate intracellular parasite.



## ⇒ Bacterial viruses (Bacteriophage/ phage)

### \* Temperate phage

• lytic & lysogenic lifecycle

• Eg.  $\lambda$ -phage of E. coli

$\beta$ -phage of C. diphtheriae

### Virulent phage

• lytic lifecycle

• Eg. T<sub>4</sub>/T<sub>5</sub>

## ⇒ $\lambda$ phage

- DNA virus

- It is one of the very commonly used phage as a cloning vector

### → Characteristics:

- It is a DNA virus

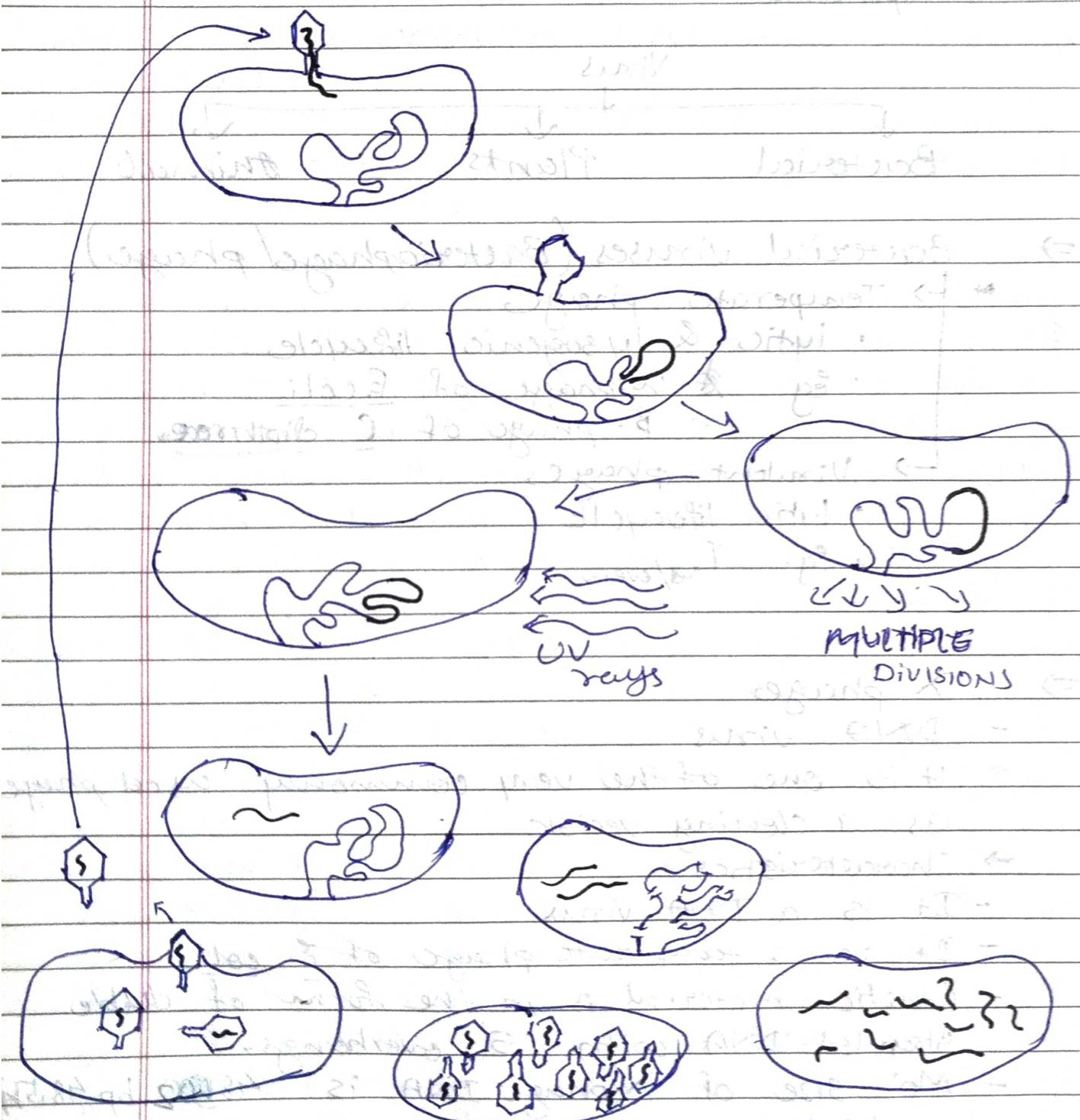
- It is a temperate phage of E. coli

↳ Genetic material is in the form of double stranded DNA with 5' overhangs.

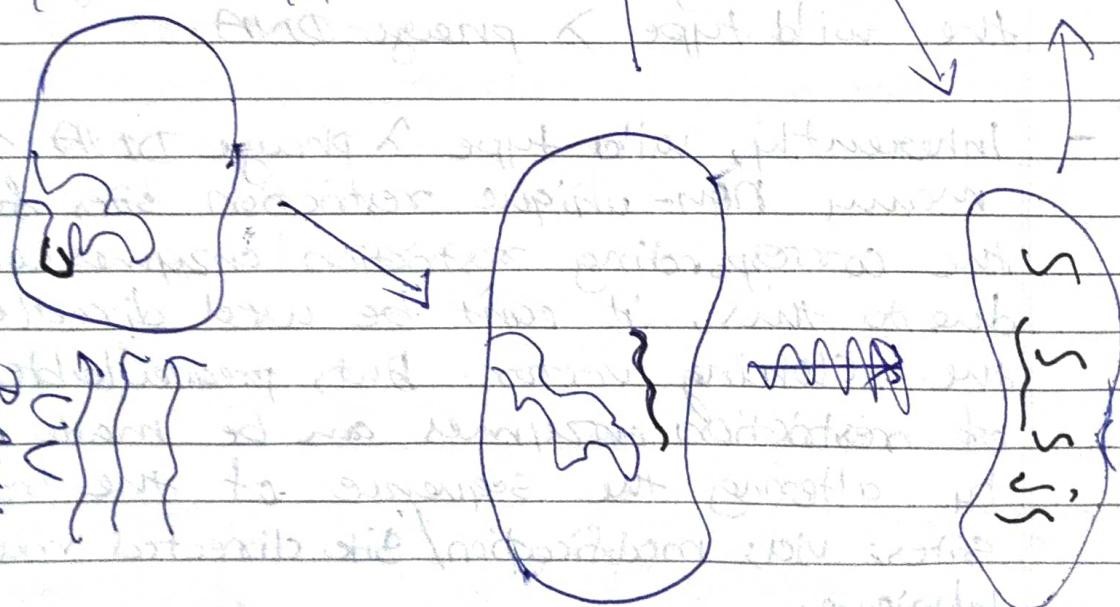
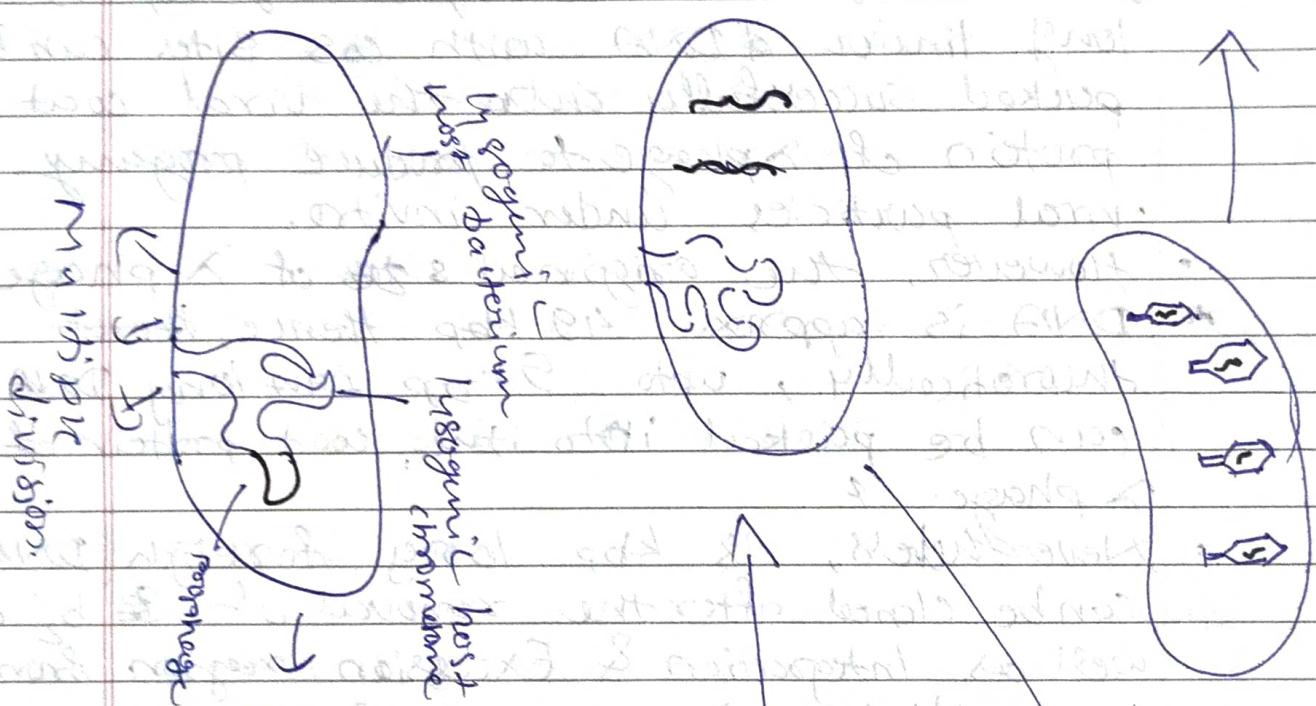
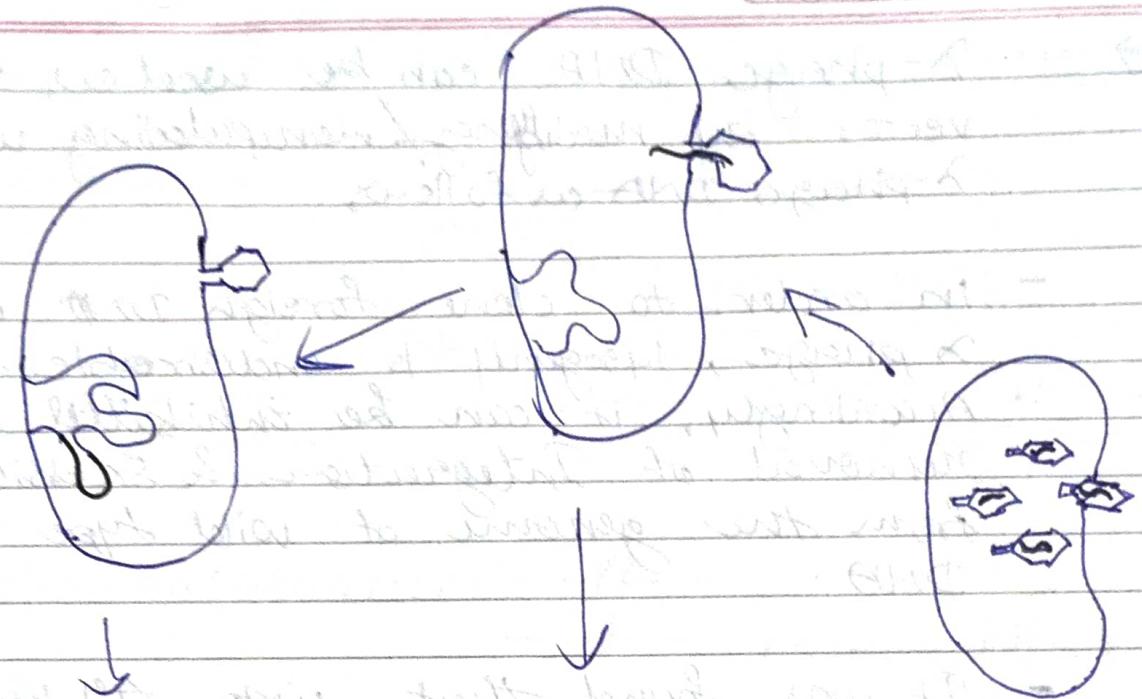
- Mol. size of  $\lambda$  phage DNA is 48502 bp or 48.5 kb.

- The 3' overhangs are 12 nucleotide longer & often known as cos sites or sticky ends.
- Cos sites are complementary to each other
- The virus shows complex bivalve symmetry.

⇒ Life cycle of  $\lambda$ -phage



Excretion and excretory system  
and the excretory system of human body

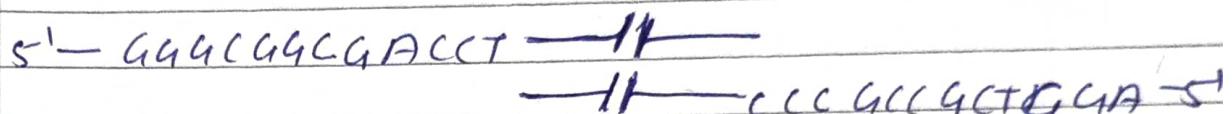
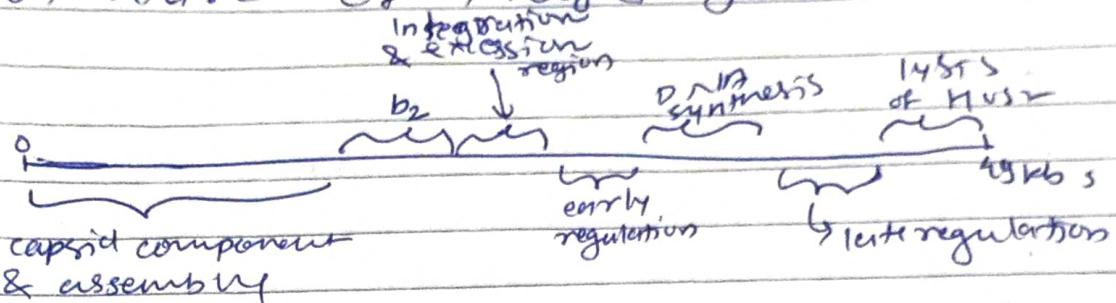


→  $\lambda$ -phage DNA can be used as cloning vector by modifying/manipulating wild type  $\lambda$ -phage DNA as follows:

- In order to clone foreign DNA using  $\lambda$  phage, lysogeny is undesirable.
- Accordingly, it can be inhibited by the removal of Integration & Excision region from the genome of wild type  $\lambda$  phage DNA.
- It was found that upto 49 kbp - 54 kbp long linear DNA with cos sites can be packed successfully into the viral coat protein of  $\lambda$  phage to produce progeny viral particles under *in vitro*.
  - However, the original size of  $\lambda$  phage DNA is approx. 49 kbp. Hence, theoretically, upto 5 kbp can long DNA can be packed into the coat protein of  $\lambda$  phage.
  - Nevertheless, 18 kbp long foreign DNA can be cloned after the removal of  $\lambda b_2$  as well as Integration & Excision region from the wild type  $\lambda$  phage DNA.
- Inherently, wild type  $\lambda$  phage DNA contains many non-unique restriction sites for the corresponding restriction enzymes within; due to this, it can't be used directly as the cloning vector. But, preavailable sites of restriction enzymes can be made defective by altering the sequence of the restriction sites via: modification/site directed mutagenesis technique.

This should be followed by addition of MCS, or polylinkers within the  $\lambda$  phage DNA.

$\Rightarrow$  Structure of  $\lambda$  Phage genome



$\Rightarrow$

$\lambda$  phage genome based vectors

- $\hookrightarrow$   $\lambda$  insertion vector
- $\hookrightarrow$   $\lambda$  replacement vector

$\Rightarrow$   $\lambda$  insertion vector

- Obtained by deleting  $b_2$  region from wild type  $\lambda$  phage DNA.
- Eg.  $\lambda$  gt10 &  $\lambda$ ZAPII

$\rightarrow$   $\lambda$ gt10

- C<sub>r</sub> repressor gene in between Integration & Excision region helps in formation of  $\lambda$  repressor protein
  - $\lambda$  repressor protein is important for establishment of lysogeny
  - EcoRI site - 5'- GAATTC - 3'
  - cloning of foreign DNA
- $\frac{\text{foreign DNA}}{\text{phage DNA}}$
- ↓ RB  $\rightarrow$  Eg. EcoRI

DNA Ligase  $\downarrow$  (foreign DNA)



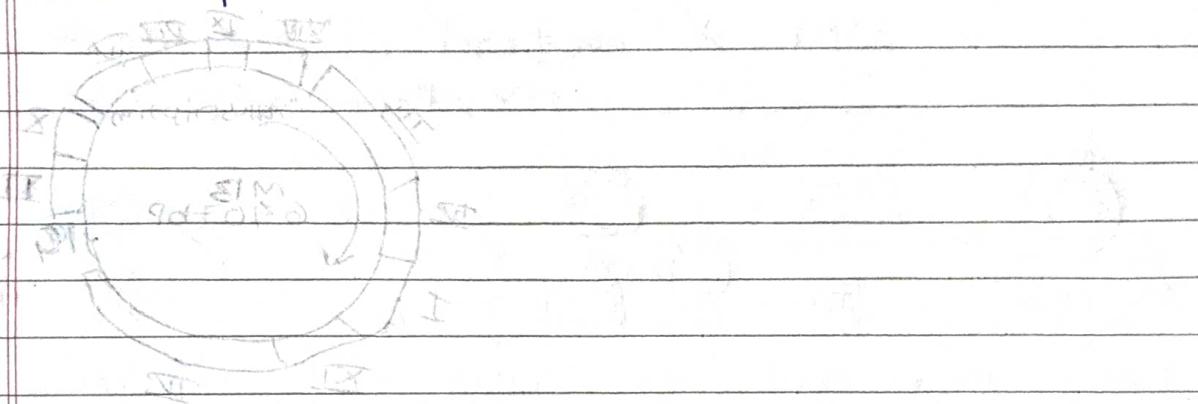
few recombinant & few self-ligated  $\lambda$  gt10

$\downarrow$  in vitro Recombining

$\rightarrow$  infect th

$\rightarrow$  cell

- ⇒ M13 Phage to work with
- Single stranded circular DNA
  - 6407 bp long
  - virulent phage.
  - 507 kbp long intergenic sequence with unique & 2 unique restriction site in the replicative form of M13 ds DNA.
  - 36 kbp of DNA can be inserted.



most suitable for major biotechnology

→ Replicative end DNA and SIM to

replicate & produce phage particles.

can form recombinant DNA.

but anti-restriction enzyme cannot be

removed in such SIM on horizon

large segment can get off

another problem linked gene SIM

1 gm SIM ←

2 gm SIM ←

3 gm SIM ←

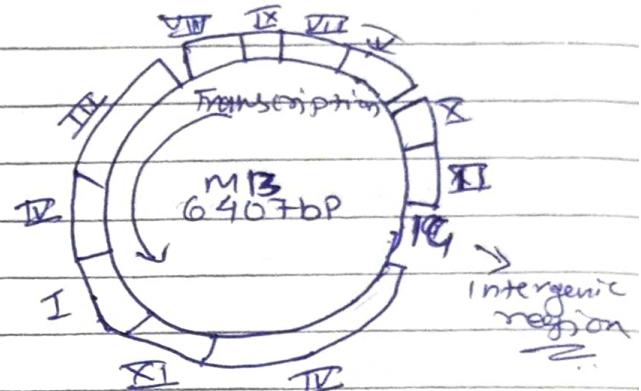
1 gm SIM ←

both genes is very less and small =

extra 2-3 genes may be 20 million =

but still there is

- ⇒ Replicative form of M13 DNA.
- ~ 6.4 kbp
  - ~ 11 genes
  - All are essential
  - ~ 50% base pairs modifiable
  - → contains Intergenic region for addition of DNA  
→ has ori site
  - Restriction sites for AsuII & Avall



Intergenic region of replicative form of M13 DNA can be manipulated without disturbing ori site & foreign DNA can be cloned whose mol. size is ~~is~~ 6 times greater than that of original ~~is~~ M13 DNA i.e. approx 36 kbp long foreign DNA.

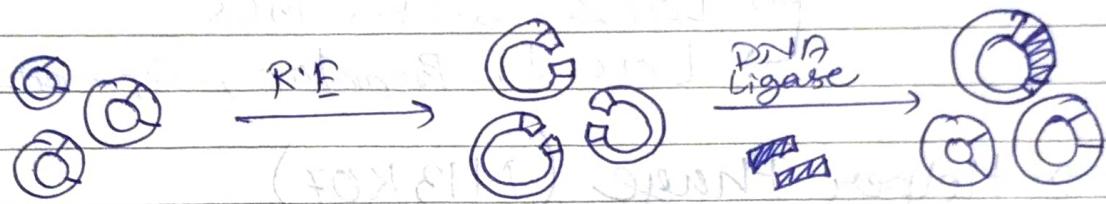
- ⇒ M13 phage DNA based Cloning vectors
- M13 mp1
  - M13 mp2
  - M13 mp7
- M13 mp1
- lac Z' gene is inserted
  - within lac Z' gene sequence 5'-GAATTG- is present/inserted.

→ M13mp2

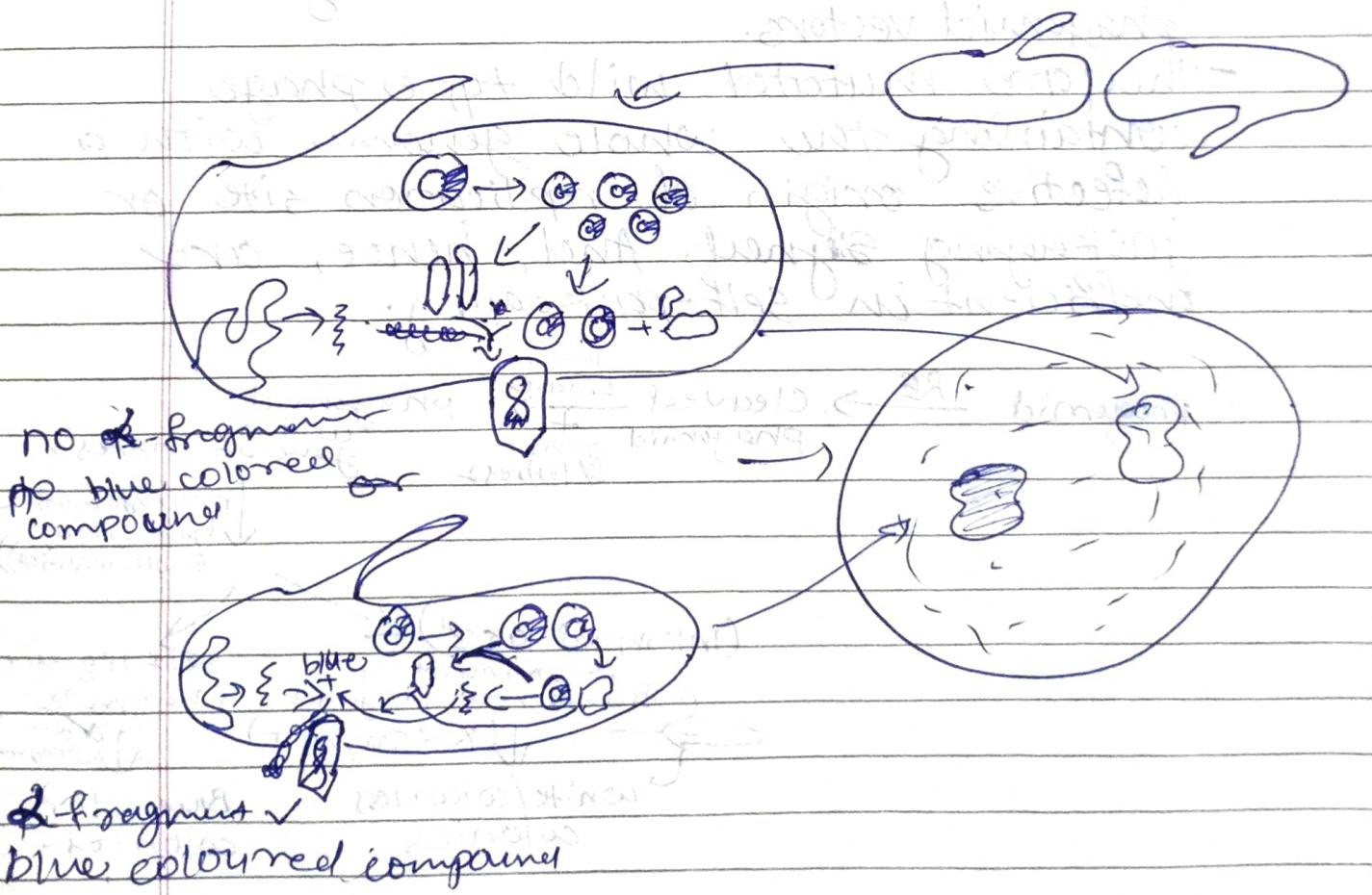
- Derivative of M13mp1
- Lac Z' gene is present but the sequence has changed to 5'-GAATTC-3'

→ M13mp7

- Derivative of M13mp2
- Lac Z' gene is present but the sequence is broken & MCS is inserted in between them.



Transformation  
CaCl<sub>2</sub> ↓ E. coli



- Advantages & Disadvantages of M13 vectors
- upto 36 kbp of foreign DNA can be incorporated.
  - disadvantage is that we get only the single strand of DNA (Gene of Interest) & have to undergo processes to form dsDNA.

→ Phagemid (pEMBL 8)

↳ pUC plasmid + Ori of M13

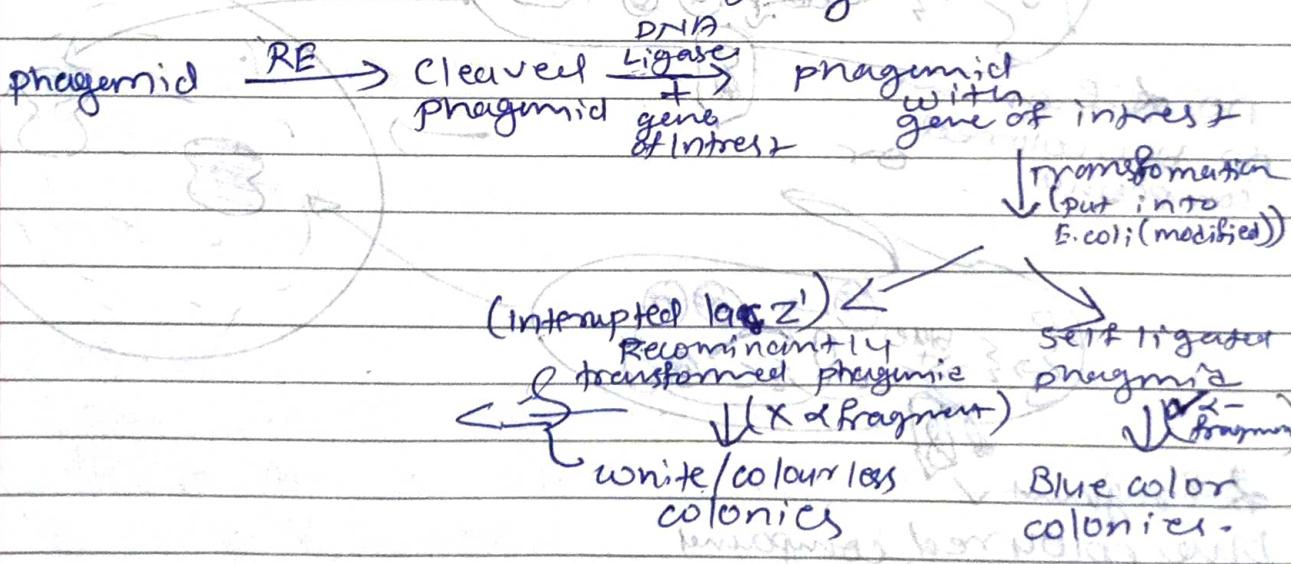
↳ bla gene (Amp<sup>r</sup> gene), Ori of E. coli

↳ LacZ' with MCS

↳ LacI, Promotor, Operator.

→ Helper Phage (M13KO7)

- Provides all necessary gene products for particle formation when using the phagemid vectors.
- They are mutated wild type phage containing the whole genome with a defective origin of replication site or packaging signal. And, hence, are inefficient in self-packing.



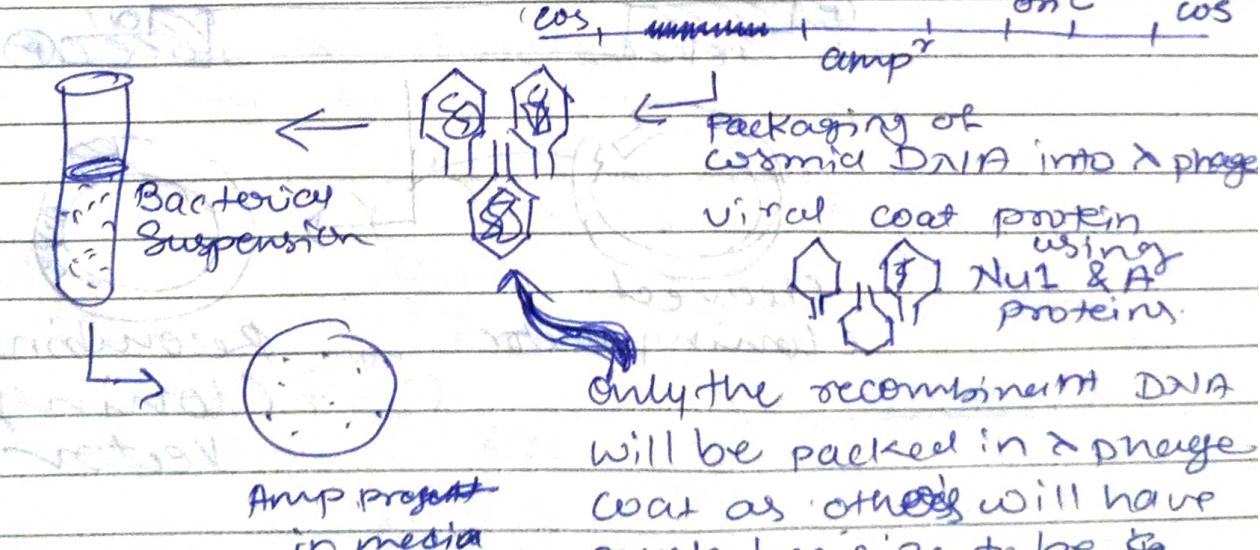
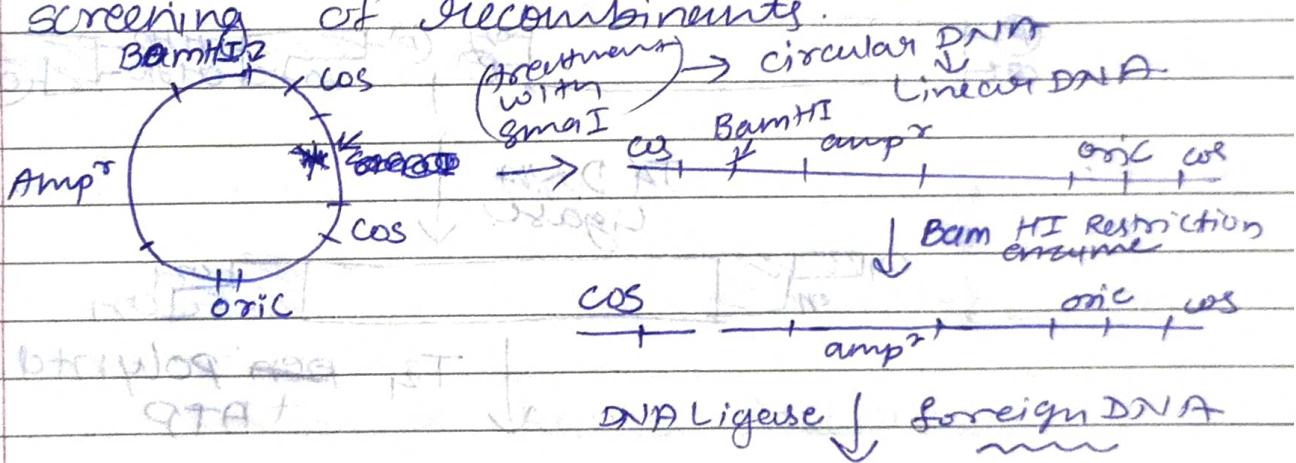
## ⇒ Cosmid

- Plasmid + λ phage cos sites
- 12 bp long overhangs of DNA.
- Origins & Antibiotic resistant genes
- Has unique restriction sites for corresponding restriction enzymes.
- Mol. Size is ~ 5-7 kb
- But, capacity to clone foreign DNA is ~ 30-45 kb.

## → Examples of Cosmid DNA

- pJB8 (~5.4 kb, amp<sup>r</sup> gene)
- pHCT9 (~6.5 kb, amp<sup>r</sup> & Tet<sup>r</sup> gene)
- C2xB (~6.8 kb, amp<sup>r</sup> & kan<sup>r</sup> gene)
- pLFR5 (~6 kb, Tet<sup>r</sup> gene)

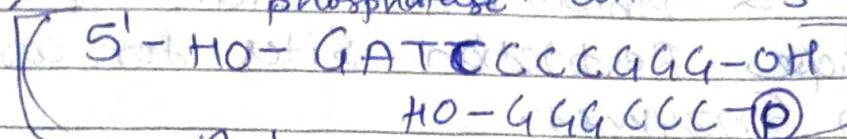
## → Use of Cosmid as cloning vector & screening of recombinants.



## ~~Blunt Ended Ligation~~

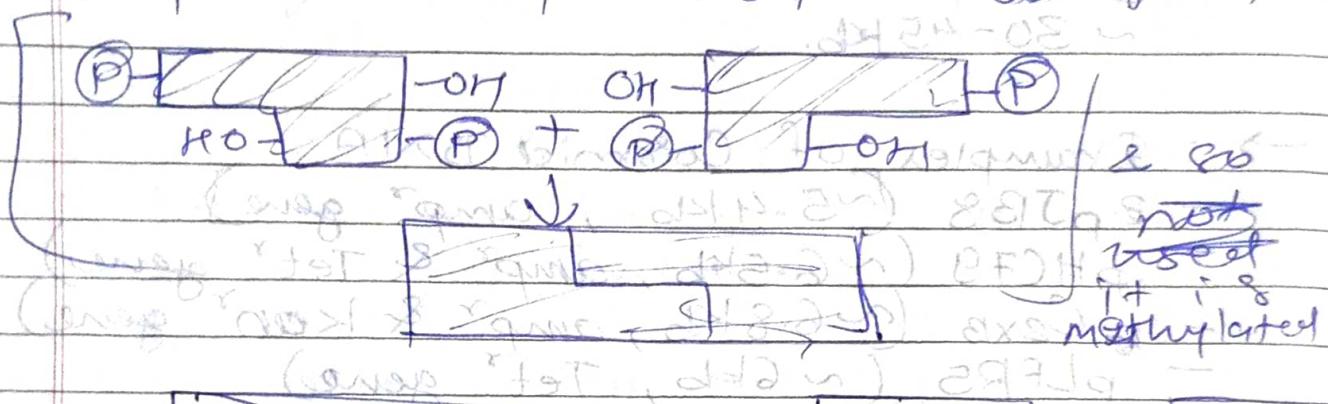
→ modified version of polylinker

⇒ Adaptors → blunt cut one end & sticky @ other  
→ treated with alkaline phosphatase to convert  $5'-\text{PO}_4-$  to  $5'-\text{OH}-$

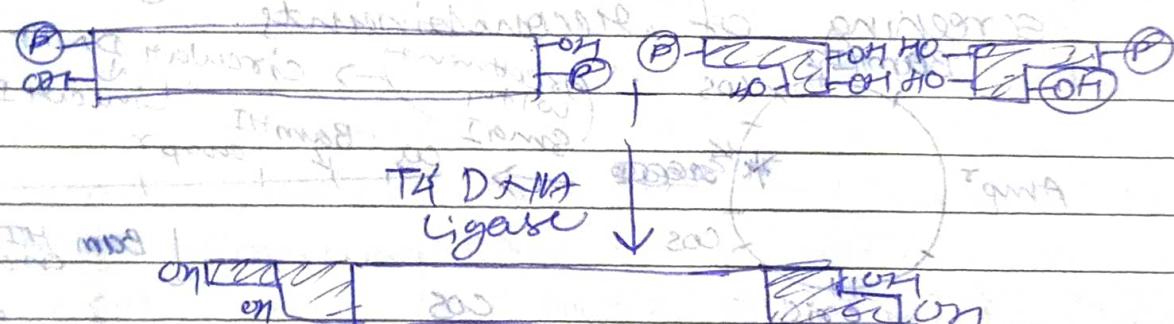


Adaptor → essential

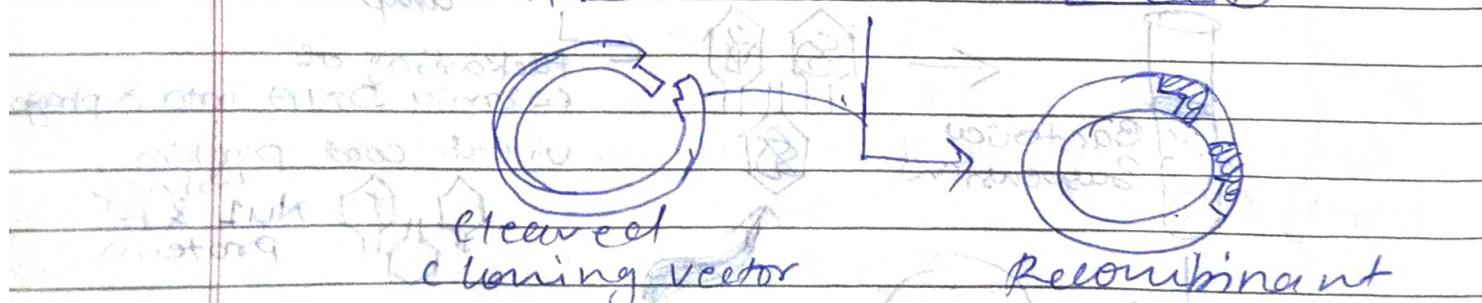
to prevent adaptor-adaptor coupling



3' methyl groups bind to 5'



T4 RNA polymerase + ATP



Recombinant Cloning Vector

→ Plasmid →  $\sim 10 - 15 \text{ kb}$

✓ Phage →  $\sim 23 \text{ kb}$

M13 phage →  $\sim 36 \text{ kb}$

Phagemid → ds & ss

Cosmid →  $\sim 45 \text{ kb}$

BAC →  $\sim 500 \text{ kb}$

### Bacterial Artificial Chromosome

⇒ BAC:

→ Properties

- Four genes (PcrA, B & C),

↳ ~~Site~~ ensures equal division of plasmid in ~~F+~~ ~~F+~~ F<sup>r</sup> cells.

- Mol. size of DNA is  $\sim 6.7 \text{ kb}$  but can carry a foreign DNA of  $\sim 500 \text{ kb}$

- contains — Ori site

— repB gene → to synthesize its

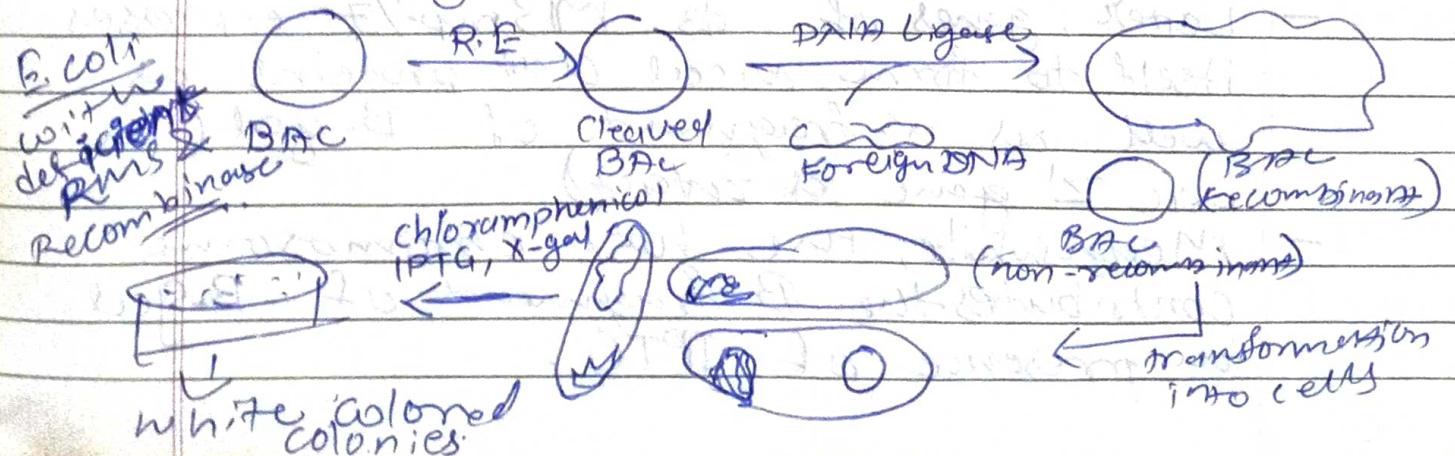
— Antibiotic resistant (AbaR) Polymerase gene

— LacI, promoter, Operator & LacZ' gene with MCS

→ created by scientist Met simon et al.

↳ and team/co-workers.

→ Cloning of foreign DNA in BAC & screening of recombinants.



⇒ Cloning of foreign DNA using M13 based cloning vectors & screening of recombinants.

- When intact ds circular DNA M13mp2, ~~M13mp7~~ is treated with suitable restriction enzyme, cleavage of cloning vector takes place & 'lacZ' gene will be interrupted.
- Such cleaved cloning vectors are then ligated with foreign DNA in presence of DNA ligase with req<sup>d</sup> cofactors to obtain recombinant as well as non-recombinant M13mp2/7.
- This is followed by the process of transformation using  $F^+$  ~~left~~ cells of E. coli which are genetically manipulated to encode only B-fragment of B-galactosidase.
- Each treated cells along with exogenous DNA are then subjected to grow on a nutrient media having ~~IPTG~~ - gal
- Non-recombinantly transformed cells possessing self-ligated M13mp2/7 show rolling circle method of DNA replication followed by loop-rolling circle method as well. This gives rise to production of mainly ds & ss circular copies of non-recombinant M13mp2/7 DNA.
- Later, genes of ds M13mp2/7 expresses itself to form viral coat proteins as well as  $\alpha$ -fragment of B-gal (as 'lacZ' gene is intact)
- Meanwhile, the host chromosome contributes the B-fragment of B-gal in presence of IPTG.

- Within a cell,  $\alpha$ - &  $\beta$ -fragments combine to form functional  $\beta$ -gal & convert taken X-gal into indigo.
- In the same line, the non-recombinant ss circular M13mp2/7 DNA undergoes packaging into the coat protein of M13 to form in numbers of progeny viral particles.
- Such viral particles come out of the blue coloured host cell via channel formation & give rise to Blue coloured turbid plaque.
- - Here, recombinantly transformed cells having recombinant M13mp2/7 show rolling & loop-rolling circle<sup>method</sup> of DNA replication to form ds & ss copies of M13 DNA.
- Genes of ds rM13mp2/7 expresses itself to produce viral coat proteins & fusion proteins (as lac Z' gene got interrupted due to the presence of foreign DNA).
- Host chromosome contributes  $\beta$  fragment of  $\beta$ -gal in presence of IPTG.
- Never the less, the functional  $\beta$ -gal is unavailable & hence, X-gal remains as X-gal.
- Meanwhile, ss rM13mp2/7 circular DNA undergoes packaging into the M13 coat proteins to produce recombinant progeny viral particles.
- Such viruses come out of the bacterium via channel formation & gives rise to white turbid plaque.
- In this exp., white turbid plaque is of our interest & hence, it can be removed out

from the media & treated further to recover recombinant viral particles from it under *in vitro* cond'n.

- Such viral particles can be reinfected to the host cell for subculturing or can be stored in refrigerator for period of time.

⇒ **YAC** [Yeast Autosome Chromosome]

- Mol. size = 10kb
- ARS1 (Autonomously replicating sequence)
- Tetomeric sequences
- Centromeric region from chromosome no. 4
- Trp1 gene → Tryptophan
- URA3 gene → Uracil
- one or more unique restriction sites.
- capacity of YAC is ~1000 kb long sequence of foreign DNA.

⇒ **Phagemid**: a hybrid of phage & plasmid

- It is combination of plasmid with ori site of M13 genome. E.g. pEMBL8
- pEMBL8 consists of pUC series plasmid & ori site of M13.

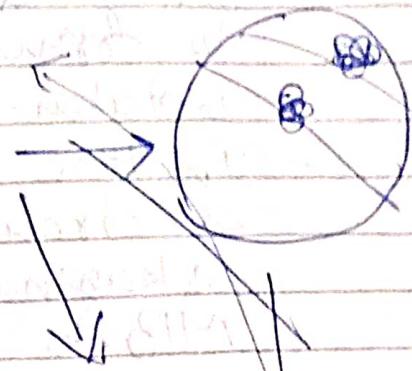
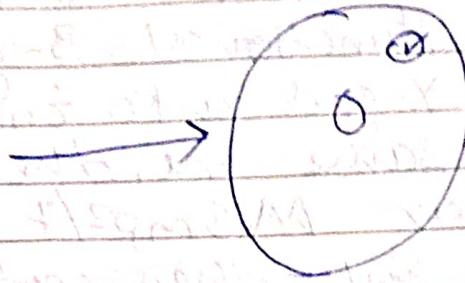
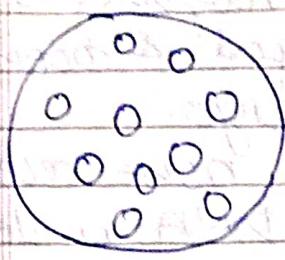
⇒ Cloning of foreign DNA in pEMBL8 & screening of recombinants

- When intact, ds circular pEMBL8 is treated with suitable R.E; *lac Z'* gene are interrupted & the cloning vector becomes linear.
- It can be ligated with foreign DNA in.

presence of DNA ligase to form transformed & non-transformed circular ds pEMBL8.

- Such ~~pEMBL8~~ recombinant vectors can later be transformed into suitable prototrophic cells like E. coli by CaCl<sub>2</sub> method (here, E. coli are genetically modified w.r.t. lacZ' gene).
- Obtained cells are later allowed to grow in the nutrient media containing IPTG, ampicillin, kanamycin.

- Resulting bacterial colony of transformed cells can be readily distinguished into recombinantly transformed & non-recombinantly transformed in nature as per the blue-white screening.
- Speciality of pEMBL8 is such that, it'll replicate as a plasmid in E. coli but it can be packaged as ~~ss~~ ssDNA into the coat protein of M13 phage - in presence of helper phage (M13KO7).
- M13KO7 phage when, monolayer culture of treated cells ~~are~~ of infected F+ genetically manipulated E. coli are infected with Helper M13 phage, it results into production of Blue coloured turbid plaque & white coloured turbid plaque.
- Blue plaques belongs to non-recombinant viral particles having non-recombinant ~~ss~~ circular pEMBL8 DNA.
- However, white plaque belongs to recombinant viral particles having recombinant ss circular pEMBL8 DNA.

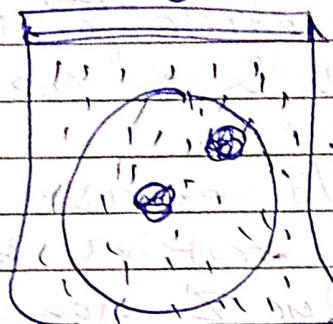


Fixation & denaturing

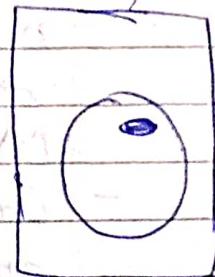
gut 20 min at  $80^{\circ}\text{C}$  N.S.

autoclaving for 2 hrs. then washing

### Hybridization



→ Autoradiogram →



⇒ Cloning of foreign DNA in recombinant & Screening of recombinants.

pJB8<sup>+</sup> is one of the commonly used cosmid in the field of PDT.

pJB8<sup>+</sup> carries Amp<sup>r</sup>, ori C [of E. coli], 2 cos sites which contains one Sma site in between & unique restriction site for BamHI.

- After the treatment of intact ds circular pJB8 with SmaI, it becomes linear.

- This is followed by treating linear pJB8 with BamHI to generate 2 separate double stranded DNAs.

- Both the fragments can be ligated together along with foreign DNA in between, in the presence of DNA Ligase.

- Here, size of the foreign DNA can be upto 45 kb.

- This process generates, recombinant pJB8 (approx. 50kb) & self-ligated pJB8 (approx. 5.4kb).

- Obtain cosmid DNA having cos sites at both the ends will be subjected for invitro packaging.

- It was found that only recombinant pJB8 will be packed into the coat protein of  $\lambda$  phage due to its high molecular size.

- However self-ligated or non-recombinant pJB8 are unable to participate in invitro packaging even after having cos sites due to its small size.

- This, clearly suggests that, invitro packaging produces only recombinant  $\lambda$  phage particles.

- Later these particles are used to infect prokaryotic cells like ampicillin sensitive E. coli.

- Whereas  $\lambda$ -phage particles adsorb on the surface of the host cell and releases  $\lambda$ -pJB8 directly into the cytoplasm of the bacteria.

- linear  $\lambda$ -pJB8 undergoes circularization, in presence of host DNA ligase.
- Such virally treated cells are able to grow under the selection pressure of ampicillin because of the presence of Amp<sup>r</sup> within  $\lambda$ -pJB8, taken cosmid now replicates independently without associating with host chromosome.
- Virally non-infected cells, lacks,  $\lambda$ -pJB8 & thereby lacks Amp<sup>r</sup> gene. And so are unable to survive in presence of ampicillin.
- obtained colonies of E. coli belonging to recombinant lambda phage can be subjected for subculturing for the further cloning of the foreign DNA on the media having ampicillin.

Ligation is carried out with (S1PF) and S1 nuclease →  
Foreign DNA (linearized) is removed & standard restriction

- Bac is one of the v. commonly used type of cloning vector in RDT.
- For the cloning of foreign DNA (500kb approx) in prokaryotic host cell.
- It is discovered by Mew & co-workers.
- mol. size of Bac is 6.7 kb approx.

→ Characteristics.

- Bac containing Par genes i.e., ParA, ParB, ParC, obtained from F-plasmid. Par gene products plays an important role in the distribution of equal no. of copies of Bac into daughter cells.
- Bac shows v. stable ori site.
- Bac possesses RepE gene → encodes DNA pol., which helps to maintains copy number of Bac.
- Bac also contains antibiotic resistance gene, LacI, promoter, operator & LacZ' gene with MCS.

→ Cloning of Foreign DNA in Bac & screening of recombinants.

- When an intact, circular ds Bac having chloramphenicol resistant gene is treated with suitable R.E., it undergoes cleavage & becomes linear.
- This is followed by ligation of cleaved Bac & foreign DNA together in presence of DNA ligase to obtain self-ligated & recombinant Bac.

- Such Bac DNA can be transformed into Prokaryotic host cell using Electroporation.
- Treated cells can be subjected to growth in nutrient media containing chloramphenicol, X-gal & IPTG.
- Under the selection pressure of chloramphenicol only the transformed cells will survive & among them, recombinantly & non-recombinantly transformed prokaryotic host cells can be easily identified via blue-white screening.
- Here, prokaryotic host cells should be deficient of RMS (Restriction Modification system) & Recombinase. Host cells should possess 'defective LacZ' gene in the chromosome encoding  $\beta$ -fragment of  $\beta$ -gal

## $\Rightarrow$ Yeast Artificial Cell (YAC)

- E. coli is one of the most suitable prokaryotic host cell used in genetic engineering & Yeast is one of the most suitable Eukaryotic host cell for the same.
- YAC is v. commonly used type of cloning vector to clone foreign DNA whose mol. size is approx. 1000 kb.
- YAC possess following DNA sequences which are essential to maintain itself within nucleus of the yeast.

they're as follows -

- **ARS1** [Autonomously replicating sequence] → A site from where DNA replication begins.
- **CN43** → centromeric region from Chromosome no. 4 of Yeast cells.
- **TEL** → Telomeric sequences are present at the end of YAC to maintain its size.
- Selectable markers
  - ↳ **Eg:** & **TRP1** → gene products → Tryptophen
  - ↳ **URA3** (gene product) → uracil biosynthesis
  - ↳ **URA3** (gene product) → uracil biosynthesis
- Mol. size of YAC is 10kb approx & contains one or more unique as well as non-unique restriction site for ~~one~~ corresponding R.P.

⇒ Cloning of Foreign DNA in YAC & Screening of recombinants.

- Treatment of intact ds circular YAC with BamHI opens up the circle & makes YAC linear
- Linearized YAC, when treated with suitable R.P. It forms, left arm & right arm.
- Both the arms can be ligated

together, along with foreign DNA in between using DNA ligases

- This generates self-ligated recombinant, linear YAC
- Transfect or Transform into the yeast spheroplast by electroporation method.
- Treated cells are then subjected to grow on a minimal media (deficient of tryptophan & uracil). As a result, only transformed/transfected yeast cells will be able to grow on minimal media.
- Note: Here, Yeast cells are inherently deficient of tryptophan & uracil biosynthesis.
- Non-recombinantly transfected yeast cells / colonies are unable to survive on minimal media after few days. as they've off taken self-ligated YAC of size  $\approx 10$  kb approx. which often undergoes disintegration.
- Among survived colonies, those which possess recombinant YAC with or without G418 can be screened with the aid of colony hybridization technique.

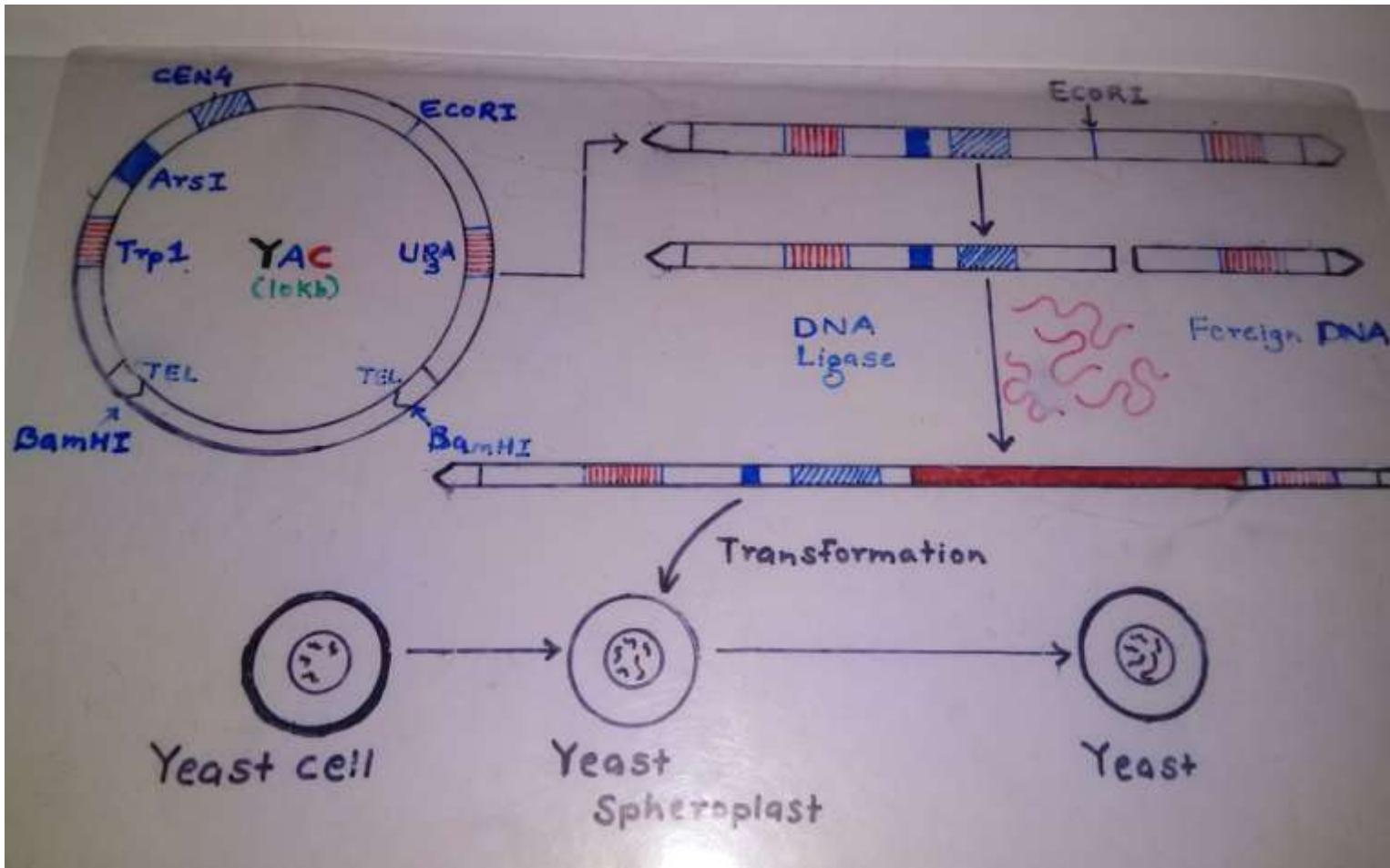
# YAC

Yeast artificial chromosome

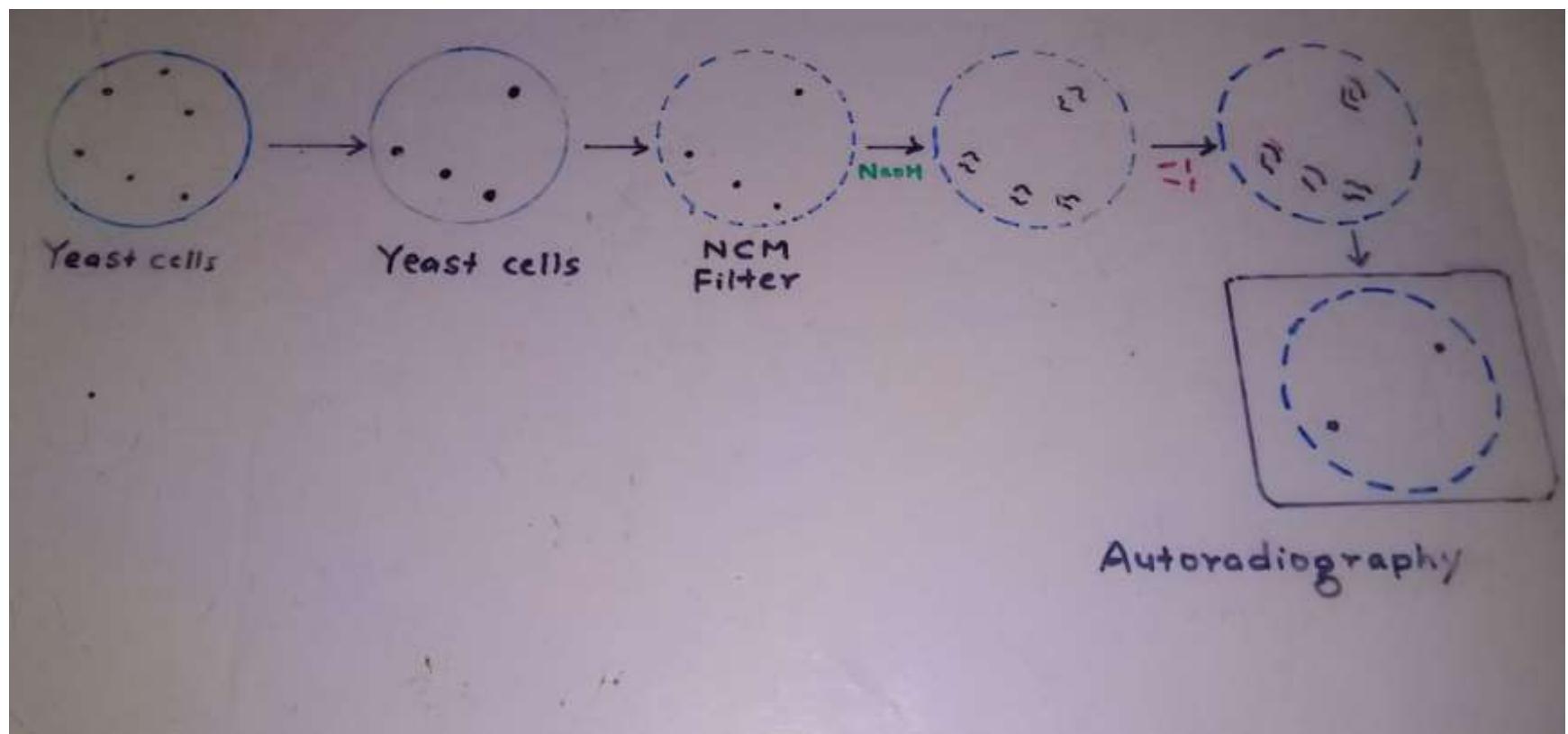
# Properties of YAC

- Molecular size of YAC is 10Kb
- ARS 1 (Autonomously replicating sequence)
- Telomeric sequences
- Centromeric region from Chr.no.4
- Trp1 gene
- URA3 gene
- One or more unique RS
- Capacity of YAC is ~1000Kb long foreign DNA

# Cloning of foreign DNA using YAC



# Screening of recombinants



# Colony hybridization

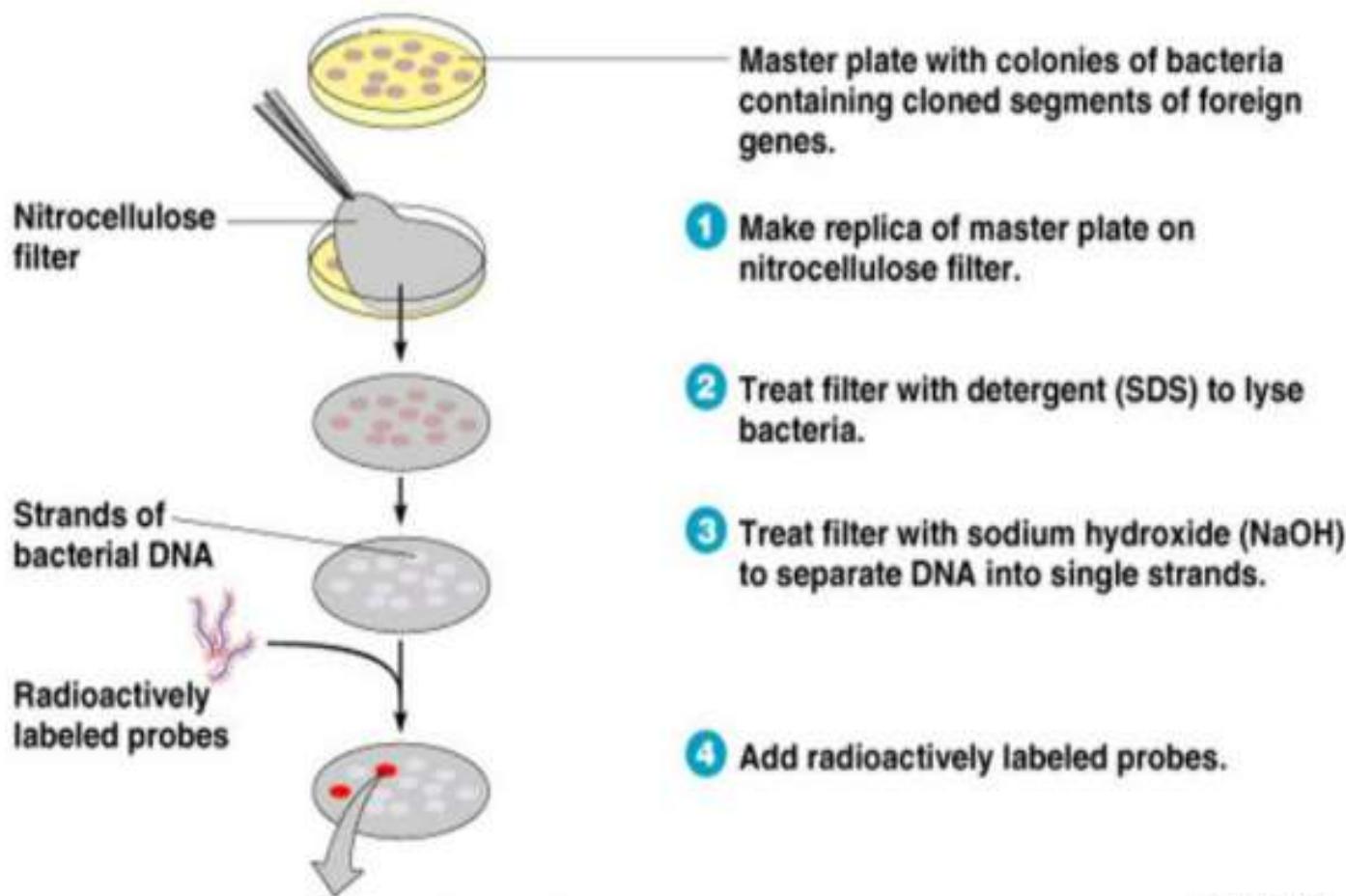


Figure 9.12.1

# Application

- Gene mapping
- Chromosome walking

спасибо

**GRACIAS**

**THANK YOU**

ありがとうございました **MERCI**

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